भारतीय मानक

सुक्रालोस, खाद्य ग्रेड — विशिष्टि

Indian Standard

SUCRALOSE, FOOD GRADE — SPECIFICATION

ICS 67.220.20

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

May 2013 **Price Group 4**

Food Additives Sectional Committee, FAD 8

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council.

Sucralose is an artificial sweetener. It is approximately 600 times sweeter than sucrose (table sugar), twice as sweet as saccharin, and four times as sweet as aspartame. Unlike aspartame, it is stable under heat and over a broad range of *p*H conditions and can be used in baking or in products that require a longer shelf life.

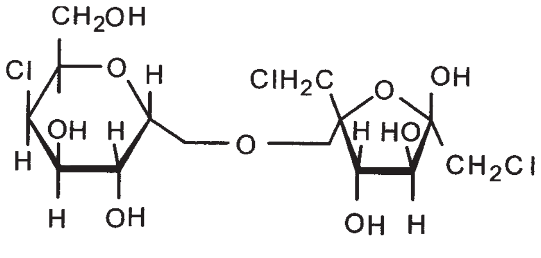
To ensure the quality of the product, it is necessary to have quality control specifications. While formulating this standard considerable assistance has been derived from JECFA standard for sucralose, published by FNP 52 Add 9 (1993) and revised at the 63rd JECFA (2004). This standard has been formulated to provide the necessary guidelines to manufacturers and the consumers for maintaining quality control.

In the formulation of this standard, due consideration has been given to the *Food Safety and Standards Act,* 2006 and the Rules framed thereunder. Due consideration has also been given to the *Legal Metrology (Packaged Commodities) Rules*, 2011. However, this standard is subject to restrictions imposed under these, wherever applicable.

Description

Common name — Sucralose
ADI — 0-15 mg/kg bw
Chemical name — 1,6-Dichloro-1,6-dideoxy-ß-D-fructofuranosyl-4-chloro-4-deoxy-alpha-D-galactopyranoside
C.A.S number — 56038-13-2
Chemical formula — $C_{12}H_{19}Cl_3O_8$
Formula weight — 397.64
Specific rotation — [alpha] 20, D: Between +84.0 and +87.5° (10% *w/v* solution)
Solubility — Freely soluble in water, methanol and ethanol; slightly soluble in ethyl acetate

Structural formula:



In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated expressing the result of test or analysis is to be rounded off, it shall be done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

SUCRALOSE, FOOD GRADE — SPECIFICATION

1 SCOPE

This standard prescribes requirements, methods of sampling and test for sucralose, food grade.

2 REFERENCES

The following standards contain provisions, which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
266 : 1993	Sulphuric acid (*third revision*)
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1995	Methods of sampling and test for food colours (*second revision*)
2362 : 1993	Determination of water by Karl Fischer method — Test method (*second revision*)
2491 : 1998	Food hygiene — General principles — Code of practice (*second revision*)

3 REQUIREMENTS

3.1 Description

Sucralose is available as white to off-white, practically odourless crystalline powder.

3.2 Infrared Absorption

The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum in Annex A.

3.3 Test for Other Chlorinated Disaccharides

It should pass the test when tested in accordance with Annex B.

3.4 Thin Layer Chromatography

The main spot in the test solution should have the same R_f value as that of the main spot of Standard Solution A obtained in the test for other chlorinated disaccharides (*see* Annex B) when tested in accordance with Annex C.

3.5 Test for Chlorinated Monosaccharides

It should pass the test when tested in accordance with Annex D.

3.6 The material shall also conform to the requirements given in Table 1.

3.7 The material shall be processed, packed, stored and distributed under hygienic conditions in licensed premises (*see* IS 2491).

4 PACKING STORAGE AND MARKING

4.1 Packing

The material shall be securely packed in well-filled containers with minimum access to air. The containers shall be such as to preclude contamination of the contents with metals or other impurities.

4.2 Storage

The material shall be stored in a cool and dry place so as to avoid excessive exposure to heat.

4.3 Marking

Each container shall be legibly and indelibly marked

Table 1 Requirements for Sucralose, Food Grade

(*Clause* 3.6)

Sl No.	**Characteristic**	**Requirement**	**Method of Test, Ref to**	
			IS No.	Annex of this Standard
(1)	(2)	(3)	(4)	(5)
i)	Assay, percent by mass on an anhydrous basis	98-102	—	E
ii)	Water, percent by mass, *Max*	2	IS 2362	—
iii)	Sulphated ash, percent by mass, *Max*	0.7	—	F
iv)	Triphenylphosphine oxide, mg/kg, *Max*	150	—	G
v)	Methanol, percent by mass, *Max*	0.1	—	H
vi)	Lead (as Pb), mg/kg, *Max*	2	Clause 15 of IS 1699	—

with the following information:

a) Name of the material including the words 'Food Grade';
b) Name and address of the manufacturer;
c) Batch or Code number;
d) Net quantity when packed;
e) Instruction for storage;
f) Best before date (Month and Year to be given by the manufacturer); and
g) Any other requirements as given under the *Legal Metrology (Packaged Commodities) Rules,* 2011 and *Food Safety and Standards (Packaging and Labelling) Regulation,* 2011 and Rules framed thereunder.

4.3.1 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

4.3.1.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

5 SAMPLING

Representatives samples of the material shall be drawn according to the method given in **4** of IS 1699.

6 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

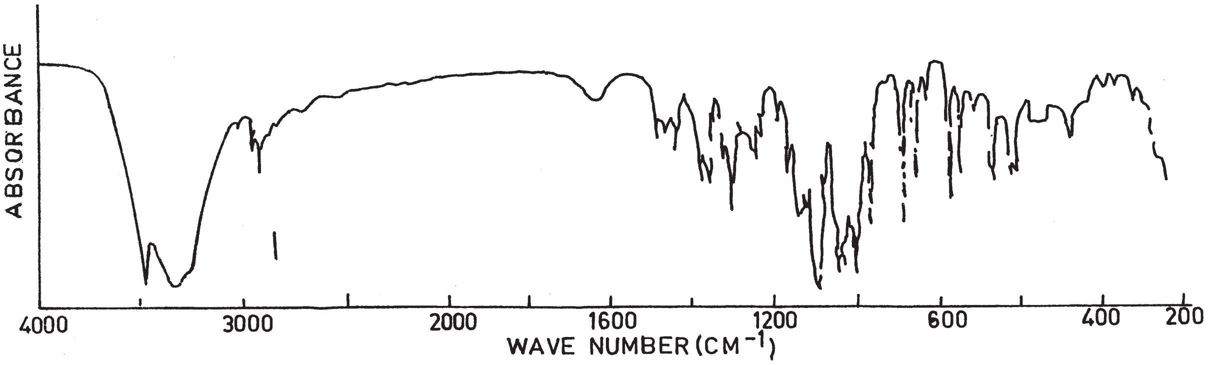
NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities, which affect the results of lest or analysis.

ANNEX A

(*Clause* 3.2)

INFRARED SPECTRUM OF SUCRALOSE

A-1 INFRARED SPECTRUM



ANNEX B

(*Clauses* 3.3 *and* 3.4)

TEST FOR OTHER CHLORINATED DISACCHARIDES

B-1 REQUIREMENTS

B-1.1 TLC Plates — Use reverse phase thin-layer chromatography plates coated with 0.20 mm layer of silica gel absorbent (for example, Whatman LKC_{18}).

B-2 REAGENTS

B-2.1 Mobile Phase — Mix 7 volumes of a 5.0 percent *w/v* aqueous solution of sodium chloride and 3 volumes of acetonitrile.

B-2.2 Spray Reagent — Use 15 percent *v/v* solution of concentrated sulphuric acid in methanol.

B-2.3 Standard Solutions — Dissolve 1.0 g of sucralose reference standard in 10 ml of methanol (Solution A). Dilute 0.5 ml of Solution A with methanol to 100 ml (Solution B).

B-2.4 Test Solution — Dissolve 1.0 g of sample in 10 ml of methanol.

B-3 PROCEDURE

Apply 5 µl each of Solution A, Solution B and test solution to the bottom of the chromatographic plate. Place the plate in a suitable chromatography chamber containing freshly prepared mobile phase and allow the solvent front to ascend 15 cm. Remove the plate from the chamber, allow it to dry and spray with spray reagent. Heat the plate in an oven at 125°C for 10 min. The main spot in the test solution has the same R_f value as the main spot in Solution A and no other spot in the test solution is more intense than the 0.5 percent spot in Solution B.

ANNEX C

(*Clause* 3.4)

THIN LAYER CHROMATOGRAPHY

C-1 PRINCIPLE

In thin layer chromatography (TLC), the stationary phase is a uniform layer of a finely divided powder that has been coated on the surface of a glass or plastic sheet and that is held in place by a binder. The capacity of the system is dependent on the thickness of the layer, which may range from 0.1 to 2.0 mm. The thinner layers are used primarily for analytical separations, while the thicker layers, because of their greater sample-handling ability, are useful for preparative work.

Substances that are used as coatings in TLC include silica gel, alumina, cellulose and reversed phase packings. Separations occur due to adsorption of the solutes from the mobile phase onto the surface of the thin layer. However, adsorption of water from the air or solvent components from the mobile phase can give rise to partition or liquid-liquid chromatography. Specially coated plates are available that permit ion-exchange or reverse-phase separations.

C-2 APPARATUS

Acceptable apparatus and materials for thin layer chromatography consist of the following.

C-2.1 Glass Plates — Flat glass plates of uniform thickness throughout their areas. The most common sizes are 20 cm × 20 cm and 5 cm × 20 cm (aluminum plates also are commonly used).

C-2.2 Aligning Tray — An aligning tray or other suitable flat surface is used to align and hold plates during application of the adsorbent.

C-2.3 Adsorbent — The adsorbent may consist of finely divided adsorbent materials for chromatography. It can be applied directly to the glass plate, or it can be bonded to the plate by means of plaster of Paris or with starch paste. Pretreated chromatographic plates are available commercially.

C-2.4 Spreader — A suitable spreading device that when moved over the glass plate applies a uniform layer of adsorbent of desired thickness over the entire surface of the plate.

C-2.5 Storage Rack — A rack of convenient size to hold the prepared plates during drying and transportation.

C-2.6 Developing Chamber — A glass chamber that can accommodate one or more plates and can be properly closed and sealed as described under paper chromatography. It is fitted with a plate-support rack that can support the plates when the lid of the chamber is in place.

C-2.7 Uttra Violet Chamber — A ultra violet viewing chamber with eye protection fitted with ultraviolet light source of short (254 nm) and long (360 nm) ultraviolet wavelengths suitable for observations may be required.

C-3 PROCEDURE

Clean the plates scrupulously, as by immersion in a chromic acid cleansing mixture, and rinse them with copious quantities of water until the water runs off the plates without leaving any visible water or oily spots, and then dry. Arrange the plate or plates on the aligning tray and secure them so that they will not slip during the application of the adsorbent. Mix an appropriate quantity of adsorbent and liquid, usually water, which when shaken for 30 s gives a smooth slurry that will spread evenly with the aid of a spreader. Transfer the slurry to the spreader, and apply the coating at once before the binder begins to harden. Move the spreader smoothly over the plates from one end of the tray to the other. Remove the spreader, and wipe away excess slurry. Allow the plates to set for 10 min, and then place them in the storage rack and dry at 105°C for 30 min. Store the finished plates in a desiccator.

The chromatographic chamber is sealed to allow equilibration (saturation) of the chamber atmosphere and the paper with solvent vapour. Any excess pressure is released as necessary. For large chambers overnight equilibration may be necessary.

Apply the sample solution and the standard solution at points about 1.5 cm apart and about 2 cm from the lower edge of the plate (the lower edge is the first part over which the spreader moves in the application of the adsorbent layer), and allow to dry. A template will aid in determining the spot points and the 10 cm to 15 cm distance through which the solvent front should move.

Arrange the plate on the supporting rack (sample spots on the bottom), and introduce the rack into the developing chamber. The solvent in the chamber must be deep enough to reach the lower edge of the adsorbent, but must not touch the spot points. Seal the cover in place, and maintain the system until the solvent ascends to a point 10 to 15 cm above the initial spots, this usually requiring from 15 min to 1 h. Remove the plates, and dry them in air. Measure and record the distance of each spot from the point of origin.

ANNEX D

(*Clause* 3.5)

TEST FOR CHLORINATED MONOSACCHARIDES

D-1 REQUIREMENTS

D-1.1 TLC Plates

Use a thin layer chromatography plate coated with a 0.25 mm thickness of silica gel 60 or equivalent.

D-2 REAGENTS

D-2.1 Spray Reagent

Dissolve 1.23 g *p*-anisidine and 1.66 g phthalic acid in 100 ml methanol. Store the solution in darkness and refrigerate to prevent it becoming decolourised. Discard if solution becomes discoloured.

NOTE — *p*-anisidine is toxic by skin absorption and inhalation and should be used with due caution.

D-2.1.1 *Standard Solution A* — Dissolve 10.0 g of mannitol, weighed to 0.001 g, in water in a 100 ml volumetric flask, and dilute to volume with water.

D-2.1.2 *Standard Solution B* — Dissolve 10 g of mannitol and 40 mg of fructose (Analar grade) in 25 ml of water in a 100 ml volumetric flask and make up to volume with water.

D-2.1.3 *Sample Solution* — Dissolve 2.5 g of sample in 5 ml of methanol in a 10 ml volumetric flask and make up to volume with methanol.

D-3 PROCEDURE

Spot 5 µl of each of standard Solutions A and B onto the TLC plate applying the solution slowly in 1 µl aliquots and allowing the plate to dry between applications. Spot 5 µl of the sample solution onto the plate in a similar manner. The three spots should be of similar size. Spray the plate with spray reagent and heat at 100 ± 2°C for 15 min. Immediately after heating, view the plate against a dark background. The spot from the sample solution is not more coloured than the spot from Solution B (Equivalent to a limit of 0.1 percent maximum total chlorinated monosaccharides) (Darkening of the mannitol spot from standard Solution A indicates that the plate has been held too long in the oven, and a second plate should be prepared).

ANNEX E

[*Table* 1, *Sl No.* (i)]

ASSAY OF SUCRALOSE

E-1 CHROMATOGRAPHIC SYSTEM

Fit a high pressure liquid chromatograph, operated at room temperature, with a radial compression module containing a 10 cm 5 µm C_{18} reverse phase column. The mobile phase is maintained at a pressure and flow rate (typically 1.5 ml/min) capable of giving the required elution time (*see* **E-5**). An ultraviolet detector that monitors absorption at 190 nm, or a refractive index detector is used.

E-2 MOBILE PHASE

Add 150 ml of acetonitrile (HPLC grade, far UV, filtered through a 0.45 µm millipore filter or equivalent) to 850 ml of water (glass distilled, filtered through a 0.45 µm millipore filter or equivalent). Mix and degas thoroughly.

E-3 STANDARD SOLUTION

Weigh accurately about 250 mg of sucralose reference standard into a 25 ml volumetric flask. Dissolve and make up to volume using the mobile phase. Filter the solution through a 0.45 µm millipore filter or equivalent. Record the weight of reference standard as W_s.

E-4 TEST SOLUTION

Weigh accurately about 250 mg of sample into a 25 ml volumetric flask. Dissolve and make up to volume using the mobile phase. Filter the solution through a 0.45 µm millipore filter or equivalent. Record the weight of sample as W_t.

E-5 SYSTEM SUITABILITY TEST

Inject duplicate 20 µl portions of standard solution into the chromatograph. The retention time of the sucralose should be approximately 9 min. The co-efficient of variation (100 × standard deviation divided by mean peak area) for the peak areas should not exceed 2 percent.

NOTE — The retention time quoted is appropriate for a 10 cm 5 µm Rad-Pak C_{18} column. If a column of a different make or length is used it may be necessary to adjust the proportion of acetonitrile in the eluent to obtain the required retention time.

E-6 PROCEDURE

Analyse the test solution under the conditions described above, making duplicate 20 µl injections, and calculate the mean peak area. Calculate the percentage purity from the relative peak areas of the test (A_t) and Standard Solutions (A_s) according to the following formula:

$$\text{Purity, percent} = \frac{A_t \times W_s}{A_s \times W_t} \times 100$$

Calculate the percentage purity on a water-free and methanol-free basis using the values obtained in the tests for water and methanol.

ANNEX F

[*Table* 1, *Sl No.* (iii)]

DETERMINATION OF SULPHATED ASH

F-1 REAGENT

F-1.1 Concentrated Sulphuric Acid — See IS 266.

F-2 PROCEDURE

Weigh accurately about 2 g of the material in a tared crucible. Ignite, gently at first, until the material is thoroughly charred, cool, moisten the residue with 1 ml of sulphuric acid and ignite gently till the carbon is completely consumed. Cool the crucible in a desiccator and weigh.

NOTE — Carry out the ignition in a place protected from air currents and use as low a temperature as possible to effect the combustion of carbon.

F-3 CALCULATION

$$\text{Sulphated ash percent by mass} = \frac{W_1}{W_2} \times 100$$

where

W_1 = mass of the residue, in g; and

W_2 = mass of the material taken for the test, in g.

ANNEX G

[*Table* 1, *Sl No.* (iv)]

TEST FOR TRIPHENYLPHOSPHINE OXIDE

G-1 APPARATUS

G-1.1 Chromatographic System — Typically a high-pressure liquid chromatograph, operated at room temperature, is fitted with a radial compression module containing a 5 µm C_{18} Rad-Pak reverse phase column (10 cm × 8 mm). The mobile phase is maintained at a pressure and flow rate (typically 1.5 ml/min) capable of giving the required elution time. The chromatograph is equipped with a UV detector (220 nm).

G-2 REAGENTS

G-2.1 Mobile Phase — Add 67 volumes of acetonitrile (HPLC grade, far UV, filtered through a 0.45 µm millipore filter or equivalent) to 33 volumes of water (glass distilled, filtered through a 0.45 µm millipore filter or equivalent). Mix and de-gas thoroughly.

G-2.2 Standard Solution — Weigh accurately 100 mg of triphenylphosphine oxide into a 10 ml volumetric flask. Dissolve and make up to volume using the mobile phase. Take 1.0 ml of the resulting solution and make up to 100 ml with mobile phase. From this solution prepare a further 100-fold dilution with mobile phase and use this as the standard solution. Filter through a 0.45 µm millipore filter or equivalent.

G-2.3 Test Solution — Weigh accurately about 100 mg of sample into a 10 ml volumetric flask. Dissolve and make up to volume with mobile phase. Filter through a 0.45 µm millipore filter or equivalent. Record the weight of sample as W_t mg.

G-3 PROCEDURE

Inject duplicate 25 µl portions of the standard and test solutions into the chromatograph. Under the conditions stated above the retention time for triphenylphosphine oxide is 6 min. Record the mean peak areas for the standard and test solutions as A_s and A_t respectively.

G-4 CALCULATION

Calculate the concentration of triphenylphosphine oxide (TPPO) in the sample from the following formula:

$$\text{TPPO, mg/kg} = \frac{A_t}{A_s} \times \frac{10\,000}{W_t}$$

ANNEX H

[*Table* 1, *Sl No.* (v)]

TEST FOR METHANOL

H-1 APPARATUS

Use a suitable gas chromatograph equipped with a hydrogen flame ionization detector containing a 2.1 m × 4.0 mm (id) glass column packed with Porapak PS 80-100 mesh or equivalent materials.

H-2 OPERATING CONDITIONS

The operating conditions may vary depending upon the particular instrument used but a suitable chromatogram may be obtained by using the following conditions:

a) Column temperature : 150°C (isothermal)
b) Inlet temperature : 200 °C
c) Detector temperature : 250 °C
d) Carrier gas nitrogen : 20 ml/min

H-3 REAGENTS

H-3.1 Standard Solution — Using a 2.0-ml class A volumetric pipette, pipette 2.0 ml of methanol into a 100-ml volumetric flask, dilute to volume with pyridine, and mix. Transfer 1.0 ml of this solution to a 100-ml volumetric flask, dilute to volume with pyridine, and mix.

H-3.2 Sample Solution — Weigh accurately about 2 g of the sample into a 10-ml volumetric flask, dilute to volume with pyridine, and mix.

H-4 PROCEDURE

Inject a 1-µl portion of the standard solution onto a gas chromatography column, obtain the chromatogram, and measure the area of the peak produced. The relative standard deviation for replicate injections is not more than 2.0 percent. Calculate the mean peak areas for the standard solution.

Similarly, inject a 1-µl portion of the sample solution into the gas chromatograph, and measure the areas of the peaks produced by methanol. Calculate the mean peak areas, and determine the methanol concentration using the following formula:

$$\text{Methanol, percent} = \frac{S_A \times V_S \times C_S}{A_S \times W_S}$$

where

S_A = sample area;

C_S = concentration of methanol in the standard in percent (volume of methanol × dilution factor × density of solvent equals $2 \times 10^{-4} \times 0.79 \times 100$);

V_S = volume of the sample solution;

A_S = standard area; and

W_S = weight of the sample.

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Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephones : 2323 0131, 2323 3375, 2323 9402 *Website*: www.bis.org.in

Regional Offices: *Telephones*

Central : Manak Bhavan, 9 Bahadur Shah Zafar Marg NEW DELHI 110002 — 2323 7617, 2323 3841
Eastern : 1/14 C.I.T. Scheme VII M, V. I. P. Road, Kankurgachi KOLKATA 700054 — 2337 8499, 2337 8561, 2337 8626, 2337 9120
Northern : SCO 335-336, Sector 34-A, CHANDIGARH 160022 — 260 3843, 260 9285
Southern : C.I.T. Campus, IV Cross Road, CHENNAI 600113 — 2254 1216, 2254 1442, 2254 2519, 2254 2315
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